Parasympathetic withdrawal increases heart rate after 2 weeks at 3454 m altitude

Siebenmann, Christoph; Rasmussen, Peter; Hug, Mike; Keiser, Stefanie; Flück, Daniela; Fisher, James; Hilty, Matthias P; Maggiorini, Marco; Lundby, Carsten

DOI:
10.1113/JP273726

Document Version
Peer reviewed version

Citation for published version (Harvard):
Siebenmann, C, Rasmussen, P, Hug, M, Keiser, S, Flück, D, Fisher, JP, Hilty, MP, Maggiorini, M & Lundby, C 2017, 'Parasympathetic withdrawal increases heart rate after 2 weeks at 3454 m altitude', The Journal of Physiology, vol. 595, no. 5, pp. 1619-1626. https://doi.org/10.1113/JP273726

Parasympathetic withdrawal increases heart rate after two weeks at 3,454 m altitude

Christoph Siebenmann[1,2], Peter Rasmussen[1,3], Mike Hug[1], Stefanie Keiser[1], Daniela Flück[1], James P. Fisher[4], Matthias P. Hilty[5], Marco Maggiorini[5] and Carsten Lundby[1]

[1]Center for Integrative Human Physiology, Institute of Physiology, University of Zürich, Switzerland; [2]Department of Environmental Physiology, School of Technology and Health, Royal Institute of Technology, Solna, Sweden; [3]H. Lundbeck A/S, Valby, Denmark; [4]School of Sport, Exercise & Rehabilitation Sciences, College of Life & Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK; [5]Intensive Care Unit, University Hospital of Zürich, Switzerland

Running title: Regulation of tachycardia in chronic hypoxia

Key words: Autonomic nervous system, Hypoxia, Sympathetic

Address for correspondence: Dr. Christoph Siebenmann,
Department of Environmental Physiology,
KTH Technology and Health,
Berzelius väg 13,
171 65 Solna,
Sweden
Phone: +46 (0)8 524 839 65;
Fax: +46 (0)8-33 09 23.

Category: Cardiovascular

Key points summary

- Heart rate is increased in chronic hypoxia and we tested whether this is the result of increased sympathetic nervous activity, reduced parasympathetic nervous activity, or a non-autonomic mechanism.
- In seven lowlanders, heart rate was measured at sea level and after two weeks at high altitude after individual and combined pharmacological inhibition of sympathetic and/or parasympathetic control of the heart.
- Inhibition of parasympathetic control of the heart alone or in combination with inhibition of sympathetic control abolished the high altitude-induced increase in heart rate.
- Inhibition of sympathetic control of the heart alone did not prevent the high altitude-induced increase in heart rate.
- These results indicate that a reduced parasympathetic nervous activity is the main mechanism underlying the elevated heart rate in chronic hypoxia.

Abstract

Chronic hypoxia increases resting heart rate (HR), but the underlying mechanism remains incompletely understood. We investigated the relative contributions of the sympathetic and parasympathetic nervous systems, along with potential non-autonomic mechanisms, by individual and combined pharmacological inhibition of muscarinic and/or β-adrenergic receptors.

In seven healthy lowlanders, resting HR was determined at sea level (SL) and after 15-18 days of exposure to 3,454 m high altitude (HA) without drug intervention (CONT) as well as after intravenous administration of either propranolol (PROP), glycopyrrolate (GLYC), or PROP and GLYC in combination (PROP+GLYC).

Circulating norepinephrine concentration increased from 0.9±0.4 $nmol^{-1}$ at SL to 2.7±1.5 $nmol^{-1}$ at HA ($p=0.03$). The effect of HA on HR depended on the type of autonomic inhibition ($p=0.006$). Specifically, HR was increased at HA from 64±10 to 74±12 beats min^{-1} during CONT ($p=0.007$) and from 52±4 to 59±5 beats min^{-1} during PROP ($p<0.001$). In contrast, HR was similar between SL and HA during GLYC (110±7 and 112±5 beats min^{-1}, $p=0.28$) and PROP+GLYC (83±5 and 85±5 beats min^{-1}, $p=0.25$).

Our results identify a reduction in cardiac parasympathetic activity as the primary mechanism underlying the elevated HR associated with two weeks of exposure to hypoxia. Unexpectedly, the sympathoactivation at HA that was evidenced by increased circulating norepinephrine concentration had little effect on HR, potentially reflecting down-regulation of cardiac β-adrenergic receptor function in chronic hypoxia. These effects of chronic hypoxia on autonomic control of the heart may concern not only HA dwellers, but also patients with disorders that are associated with hypoxemia.

Abbreviations

CaO_2, arterial oxygen content; CONT, control; GLYC, glycopyrrolate; HA, high altitude; HR, heart rate; PROP, propranolol; PROP+GLYC, propranolol and glycopyrrolate in combination; SL, sea level

Introduction

Acute exposure to hypoxia accelerates resting heart rate (HR) by facilitating sympathoactivation and parasympathetic withdrawal (Koller *et al.*, 1988; Siebenmann *et al.*, 2015b). As hypoxic exposure extends, HR remains elevated despite the progressive restoration of arterial O_2 content (CaO_2) that occurs with acclimatization (Wolfel *et al.*, 1994; Hansen & Sander, 2003; Naeije, 2010). The persistent activation of the sympathetic nervous system that accompanies chronic hypoxia (Hansen & Sander, 2003) may seem an obvious explanation for the elevated HR. Nevertheless, pharmacological inhibition of β-adrenergic receptors did not abolish the increase in HR associated with two weeks of exposure to high altitude (HA) (Hughson *et al.*, 1994; Wolfel *et al.*, 1994), implying a contribution of sustained parasympathetic withdrawal. Surprisingly, however, administration of muscarinic receptor antagonists induced a more pronounced increase in HR after 9 weeks at HA than at sea level (SL), indicating increased parasympathetic activity (Boushel *et al.*, 2001). These conflicting observations could relate to methodological differences, most notably the inhibition of different receptor types, as well as different subject groups and HA exposure protocols. The interpretation is further complicated since all these studies inhibited only one receptor type, requiring divergent analytical approaches to assess the respective contributions of the sympathetic and parasympathetic nervous system to the increased HR. Another explanation could be that an unknown, non-autonomic mechanism contributes to the increased HR in chronic hypoxia. Such changes of intrinsic heart rate could be demonstrated during simultaneous inhibition of β-adrenergic and muscarinic receptors, which has to our knowledge never been conducted in chronic hypoxia.

The aim of this study was to advance our understanding of the regulation of the increased HR in chronic hypoxia by isolating the relative contributions of the sympathetic and parasympathetic nervous systems as well as of potential non-autonomic mechanisms. In seven lowlanders exposed for 15-18 days to HA, we compared HR between SL and HA

after pharmacological inhibition of either muscarinic or β-adrenergic receptors, or both receptor types in combination. Based on findings in acute hypoxia (Siebenmann *et al.*, 2015b), we hypothesized that both sympathoactivation and parasympathetic withdrawal contribute to the increased HR in chronic hypoxia, so that individual inhibition of either receptor type would not prevent the acceleration of HR at HA. We further hypothesized that full cardiac autonomic blockade would abolish the HA-induced increase in HR and hence exclude a contribution of a non-autonomic mechanism.

Methods

Ethical approval

This study was approved by the ethical committee of the Swiss Federal Institute of Technology (EK 2011-N-51) and conducted in accordance with the current version of the declaration of Helsinki. All subjects gave written and oral consent to participation.

Participants

Seven healthy, male, Caucasian lowlanders (26 ± 4 yrs; 180 ± 1 cm; 76 ± 6 kg) were recruited as study subjects. All were physically active on a recreational basis. Subjects refrained from travelling to altitudes > 2,000 m within the last four weeks before the experiments.

Protocol

This study took place at the University of Zürich, Switzerland (460 m, referred to as SL) and during a four-week sojourn at the Jungfraujoch research station in the Swiss Alps (3,454 m, referred to as HA). This station offers private bedrooms for all subjects, kitchen facilities and living space, all with normal room temperatures. Subjects were transported to HA and back to SL by train. During the HA sojourn, they preserved physical activity by hiking, mountaineering and ergometer cycling. Drinking water was always available ad libitum and subjects were instructed to maintain their habitual diets, for which they ordered the required groceries. As previously reported, both body weight and composition were maintained throughout the HA sojourn (Jacobs *et al.*, 2012). Experiments were scheduled during the last week before ascent and then after 15-18 days at HA. At both altitudes, the experiments followed the same protocol and were conducted at normal room temperature, by the same investigators, and using the same equipment: Participants reported to the laboratory on two days, separated by 2 – 4 days. On both days, a venous catheter was inserted into an antecubital vein. An additional catheter was

inserted under local anaesthesia into a radial artery on the second day only. After catheterization, subjects were placed in a semi-recumbent position. On the second day, 2 ml of arterial blood were collected and analysed in a haemoximeter (ABL 800, Radiometer, Copenhagen, Denmark). Subjects then remained still for ~ 10 min, while arterial pressure was continuously monitored on the finger by the volume clamp method (Finometer PRO, Finapres Medical Systems B.V., Amsterdam, Netherlands). HR was derived as the inverse of the inter-beat interval. Cardiac stroke volume was determined from the blood pressure waveform by a three-element model of arterial input impedance (Modelflow) incorporating age, sex, height, and weight (Wesseling *et al.*, 1993). Cardiac output was calculated as HR × stroke volume. Data was recorded at a frequency of 1 kHz (Powerlab, ADInstruments, Bella Vista, Australia). For the analysis we used the 120 successive heart beats with the lowest variation.

On the first day, measurements were conducted without receptor inhibition (CONT), and then after administration of GLYC. On the second day, measurements were performed after administration of PROP, and then after additional administration of GLYC (PROP+GLYC). Neither the investigators nor the subjects were blinded towards the drug condition.

Drug administration

GLYC was infused for 5 minutes at a rate of 2.5 µg kg^{-1} min^{-1}. Subsequently, an additional bolus of 50 µg was injected every 2 min until the HR response to the bolus was < 10 %. Receptor inhibition was thereafter maintained by continuous infusion of 0.05 µg kg^{-1} min^{-1} until termination of the measurements. PROP was infused at a rate of 15 µg kg^{-1} min^{-1} for 15 min. β-receptor inhibition was subsequently challenged by infusion of a 60 µg bolus of isoprenaline and an additional 1 mg bolus of PROP was injected every 2 min until the HR response to isoprenaline was < 10 %. A continuous administration of 0.8 µg kg^{-1} min^{-1} was thereafter maintained throughout the measurements. After completion of the PROP

experiments, GLYC was administered according to the protocol specified above, while sustaining the continuous infusion of PROP.

All drugs were administered by means of an automated infusion pump (Harvard Apparatus, Harvard Biosciences, Cambridge, UK). During GLYC, the final dose was 4.3 ± 0.5 mg at SL and 3.9 ± 0.3 mg at HA. During PROP, the final dose was 21.7 ± 1.6 mg at SL and 21.0 ± 1.9 mg at HA. During PROP+GLYC, the final doses of the two drugs were 26.7 ± 1.8 (including the dose applied in the preceding PROP experiment) and 4.6 ± 0.3 mg at SL and 25.6 ± 2.6 and 3.7 ± 0.7 mg at HA.

Venous norepinephrine

At SL as well as after 2, 10 and 26 days at HA, 5 ml of venous blood were collected. Blood compartments were separated by centrifugation and the plasma immediately frozen in liquid nitrogen and stored at -80° C. Venous norepinephrine was measured in these samples as a marker for sympathetic activity by liquid chromatography-mass spectrometry.

Blood withdrawal for other experiments

Over the course of the five weeks preceding ascent to HA a total of ~150 ml of whole blood was withdrawn for different study purposes. At HA, at total of ~120 ml of blood was withdrawn at various time points before the experiments reported here were conducted.

Statistics

To assess the effect of HA within the different drug conditions we used a mixed model for repeated measures approach, unless otherwise noted. Level of subject entered as a random effect while drug and altitude levels entered as fixed effects. For changes in venous norepinephrine concentration time from start of HA sojourn entered as fixed effect. Where applicable, Tukey's post-hoc test was used for pairwise comparison. HA-induced changes in indices of arterial oxygenation were assessed by student's t-test and

187 SAS Enterprise Guide 6 (SAS Institute Inc., Cary, NC, USA) was used for the analysis. A p-
188 value < 0.05 was considered significant and values represent means ± S.D.
189

Results

Arterial blood analysis (Table 1)

Arterial O_2 tension and oxyhaemoglobin saturation were reduced at HA ($p < 0.001$). This was, however, compensated by an increase in haematocrit ($p = 0.008$) and haemoglobin concentration ($p = 0.002$), so that CaO_2 was higher at HA than at SL ($p = 0.04$).

Heart rate (Fig. 1)

During CONT, HR was 9.7 ± 7.9 beats min^{-1} higher at HA than at SL ($p = 0.007$). This effect of HA was affected by the autonomic antagonists ($p = 0.006$). Specifically, while HR was increased at HA by 7.6 ± 4.0 beats min^{-1} during PROP ($p < 0.001$), it was only insignificantly higher than at SL during GLYC (2.3 ± 6.0 beats min^{-1}, $p = 0.28$) and PROP+GLYC (2.3 ± 5.4 beats min^{-1}, $p = 0.25$).

Haemodynamics (Fig. 2)

The effect of HA on cardiac stroke volume also depended on the type of receptor inhibition ($p = 0.04$). While cardiac stroke volume was only insignificantly lower at HA than at SL during CONT (-0.2 ml ± 19.2 ml, $p = 0.8$), a reduction was observed at HA during PROP (-23.0 ± 13.4 ml, $p < 0.001$), GLYC (-12.8 ± 11.9 ml, $p = 0.01$) and PROP+GLYC (-25.7 ± 16.1 ml, $p < 0.001$). Similarly, the effect of HA on cardiac output tended to depend on the drug condition ($p = 0.06$). While HA numerically increased cardiac output during CONT by 1.1 ± 2.2 l min^{-1} ($p = 0.2$), it reduced cardiac output during PROP by 0.8 ± 0.8 l min^{-1} ($p = 0.02$), during GLYC by 1.1 ± 1.1 l min^{-1} ($p = 0.02$) and during PROP+GLYC by 2.0 ± 1.5 l min^{-1} ($p = 0.002$).

Mean arterial pressure was increased at HA ($p = 0.001$) and this response was not affected by the autonomic antagonists ($p = 0.9$). The respective increases during CONT, PROP, GLYC, and PROP+GLYC were 9.4 ± 17.9, 10.3 ± 15.3, 16.3 ± 17.6 and 16.4 ± 16.2 mmHg, respectively.

218

219 *Venous norepinephrine*

220 Venous norepinephrine concentration was 0.9 ± 0.4 $nmol^{-1}$ at SL and similar (1.1 ± 0.5

221 nmol l^{-1}, p = 0.7) on the second day at HA. Subsequently, norepinephrine concentration

222 increased to 2.7 ± 1.5 nmol l^{-1} (p = 0.03) on day 10 and to 3.0 ± 1.2 nmol l^{-1} (p = 0.007) on

223 day 26 at HA.

224

Discussion

As expected, HR during CONT was higher at HA than at SL despite complete restoration of CaO_2. A similar HA-induced increase in HR was observed when β-adrenergic, but not when muscarinic receptors were inhibited. These results suggest that cardiac parasympathetic withdrawal persists throughout HA acclimatization and constitutes the dominating cardioacceleratory mechanism. The absence of a HA-induced increase in HR during combined inhibition of β-adrenergic and muscarinic receptors rules out a relevant contribution of a non-autonomic mechanism.

Acceleration of resting HR occurs within the first seconds of hypoxic exposure. This acute response is governed by a combination of sympathoactivation and parasympathetic withdrawal, although the respective contributions are unclear (Siebenmann *et al.*, 2015b). As hypoxic exposure extends, sympathoactivation persists or increases further (Hansen & Sander, 2003), as illustrated in the present study by circulating noradrenaline. Surprisingly, the similar HR between SL and HA during GLYC reveals that the contribution of this sustained sympathoactivation to the accelerated HR in chronic hypoxia is minor. A potential explanation could be that the chronically elevated sympathetic activity facilitates a down-regulation of cardiac β-adrenergic receptor function and/or density. This is supported by the blunted tachycardic response of humans to isoproterenol infusion after acclimatization to HA (Richalet *et al.*, 1988).

The effect of chronic hypoxia on parasympathetic activity is poorly understood, since direct measurement techniques are not available in humans. Circulating acetylcholine concentration may seem as an obvious marker for parasympathetic activity, but experimental evidence does not support this (Fujii *et al.*, 1997). Instead, spectral analysis of HR variability has been used and suggested that parasympathetic withdrawal persists even after 18 months at HA (Dhar *et al.*, 2014). However, this finding should be interpreted with caution, since parasympathetic indices of HR variability may be

253 influenced at HA by the concomitantly increased sympathetic activity and/or pulmonary
254 ventilation (Chapleau & Sabharwal, 2011). Nevertheless, the inability of PROP to prevent
255 the HA-induced increase in HR in the present and in earlier studies (Hughson *et al.*, 1994;
256 Wolfel *et al.*, 1994) supports persistent parasympathetic withdrawal in chronic hypoxia. In
257 acute hypoxia, parasympathetic withdrawal likely occurs as a reflex response to the
258 activation of pulmonary stretch receptors by enhanced ventilation (Kato *et al.*, 1988).
259 Ventilatory acclimatization facilitates further increases in pulmonary ventilation in chronic
260 hypoxia (Bender *et al.*, 1989), which may explain persisting parasympathetic withdrawal.
261 While the present results support attenuated parasympathetic activity in chronic hypoxia,
262 Boushel et al. (2001) observed that muscarinic inhibition induced a larger increase in HR
263 after 9 weeks at 5,300 m than at SL, and accordingly concluded that parasympathetic tone
264 is increased in chronic hypoxia. Since parasympathetic modulation of HR was assessed
265 without β-adrenergic inhibition, this conclusion is based on the assumption that the effect
266 of a given parasympathetic tone on HR was not affected by the severe sympathoactivation
267 that was observed at HA in these subjects (Hansen & Sander, 2003). Another obvious
268 difference to the present study is the longer exposure to more severe HA. The restored
269 CaO_2 in our subjects indicates that the most functionally important acclimatization
270 processes were completed when the experiments at HA were conducted. Accordingly, it
271 appears unlikely that parasympathetic withdrawal would have reversed to
272 parasympathetic activation at a later point of exposure. Nevertheless, the longer and
273 more severe hypoxia in the study of Boushel et al. (2001) may have increased the density
274 of cardiac muscarinic receptors and hence the bradycardic effect evoked by a given
275 parasympathetic outflow (Kacimi *et al.*, 1993). Another explanation could be that the large
276 increase in arterial pressure that was observed in that study (Calbet, 2003) enhanced
277 parasympathetic tone by activation of arterial baroreceptors. In the present study, the HA-
278 induced increase in arterial pressure was milder, presumably due to the lower altitude.
279 In another study, a larger HR response to GLYC administration than at SL was observed
280 after the same duration of HA exposure as in the present study (Bao *et al.*, 2002).

Unexpectedly, HR in the absence of receptor inhibition was not higher at HA than at SL in that study, which may explain the more pronounced difference to HR measured at HA after muscarinic inhibition. Notably, this study also applied PROP, which did not prevent the tachycardic effect of HA. This is in agreement with the present results and provides evidence that cardiac parasympathetic activity was not elevated at HA.

In order to examine whether chronic hypoxia increases HR by a non-autonomic mechanism we performed simultaneous inhibition of β-adrenergic and muscarinic receptors. Such full autonomic inhibition has previously ruled out a contribution of a non-autonomic mechanism to the increased resting HR in acute hypoxia (Siebenmann *et al.*, 2015b). Nevertheless, functional and structural cardiac remodelling occurs in lowlanders after only 10 days at HA (Stembridge *et al.*, 2014) and it was unclear whether this encompasses an increase in the intrinsic depolarization rate of cardiac pacemaker cells. Furthermore, chronic hypoxia-induced changes in arterial pH and/or electrolyte concentration (Severi *et al.*, 2002) or simply an unknown mechanism could have increased intrinsic HR independent of structural changes. The observation that HR was similar at SL and HA during PROP+GLYC, however, suggests that a non-autonomic mechanism does not increase HR in chronic hypoxia.

We unexpectedly observed that cardiac stroke volume was not reduced at HA during CONT. A decrease in stroke volume usually occurs within the first week and thereafter persists throughout HA exposure, likely due to a reduction in plasma volume (Siebenmann *et al.*, 2013). Since stroke volume decreased at HA in all other drug conditions and also without drugs at a later point of the HA sojourn (Siebenmann *et al.*, 2013), the absence of a decrease during CONT presumably reflects a type 2 error. Interestingly, the reduction in stroke volume at HA during GLYC and PROP+GLYC, where HR did not increase, confirms that a reduced diastolic filling time is not a major component of the HA-induced reduction

308 in stroke volume (Siebenmann & Lundby, 2015).
309
310 There are several methodological aspects to consider: First, it needs to be appraised
311 whether autonomic regulation of HR after two weeks of HA exposure is representative for
312 chronic hypoxia. In support, circulating norepinephrine in the present and a previous
313 study (Mazzeo *et al.*, 1994) indicate that sympathoactivation reaches a plateau within the
314 first two weeks at HA. Furthermore, the limited insight derived from spectral analysis of
315 HR variability supports that the observed withdrawal of parasympathetic activity is at least
316 qualitatively representative for chronic hypoxia (Dhar *et al.*, 2014). Whether potential
317 changes in autonomic receptor function and/or density affect autonomic regulation of HR
318 at a later point of hypoxic exposure, however, remains to be determined. Second, it needs
319 to be considered whether experimental blood withdrawal affected our study outcome. As
320 reported previously (Siebenmann *et al.*, 2013), red cell volume at the time point of the HA
321 experiments was similar to SL, suggesting that the blood withdrawal prevented the ~ 2.5 %
322 expansion in red cell volume that would have been expected at that point (Siebenmann *et*
323 *al.*, 2015a). Nevertheless, the contribution of red cell volume expansion to the restoration
324 of CaO_2 at this altitude is small compared to those of plasma volume reductions and
325 increases in arterial O_2 saturation (Siebenmann *et al.*, 2015a). This is illustrated in the
326 present study by the observation that, despite the blood withdrawal, CaO_2 was higher at
327 HA than at SL. It therefore seems unlikely that the experimental blood withdrawal exerted
328 a confounding effect. A third methodological aspect to consider concerns the use of PROP
329 and GLYC. PROP is not only a β-adrenergic antagonist but also possesses membrane-
330 stabilizing capabilities, which may contribute to its bradycardic effect (Boucher *et al.*,
331 1992). Nevertheless, since the applied doses of PROP were similar between SL and HA,
332 this membrane stabilizing effect was presumably also similar and is hence unlikely to have
333 contributed to the increased HR at HA during PROP. GLYC, on the other hand, is a non-
334 selective muscarinic antagonist. Isolation of sympathetic control of HR requires that all
335 effects of parasympathetic modulation are prevented. Since different muscarinic receptor

types occur in the human heart (Olshansky *et al.*, 2008), a non-specific muscarinic antagonist seems appropriate for this purpose. More specific studies could now be conducted to evaluate the roles of the different muscarinic receptor types in the HR response to HA. It further needs to be considered whether PROP and GLYC completely inhibited β-adrenergic and muscarinic receptors, respectively. Adequate dosing of PROP was confirmed by isoproterenol challenge, and the final doses conformed to those that evoked complete cardiac β-adrenergic inhibition in dogs (Jose & Taylor, 1969). Although muscarinic inhibition could not be challenged by an agonist, GLYC was applied until additional administration did not evoke a further tachycardic response and the final doses highly exceeded those used in related studies (Boushel *et al.*, 2001; Bao *et al.*, 2002). Finally, the absence of an effect of HA on HR during PROP+GLYC supports that inhibition of the two receptor types was adequate. Nevertheless, during both GLYC and PROP+GLYC two subjects still presented with a notable increase in HR at HA (Fig. 1) and in one case, this was the same subject. The reason for this persisting increase is unclear since these subjects had received similar doses of GLYC as the other subjects. Furthermore, circulating norepinephrine concentration does not suggest a more pronounced HA-induced sympathoactivation. It could be speculated that cardiac β-adrenergic receptor down-regulation in chronic hypoxia (Richalet *et al.*, 1988) is subject to intra-individual variability so that a tachycardic effect of the increased sympathetic activity at HA was preserved in some subjects.

A limitation of this study is the small number of subjects included; we cannot rule out that the slight numerical increases in HR at HA during GLYC and PROP+GLYC would have reached statistical significance in a larger subject cohort. Nevertheless, since the slight HR increases at HA during GLYC and PROP+GLYC were considerably smaller than those observed during CONT and PROP, they do not contradict a dominating role of parasympathetic withdrawal. A further limitation is that our study was not double-blinded. Subject blinding was, however, not possible due to the obvious side effects of

GLYC (dry mouth, difficulty to urinate). Nevertheless, HR in a similar study proved insensitive to a placebo effect (Wolfel *et al.*, 1994). Furthermore, the 120 heart beats included into the analysis were selected by our statistics software and not by a researcher. Accordingly, we are confident that blinding of either subjects or researchers would not have changed the study outcome.

In conclusion, our results suggest that parasympathetic withdrawal persists and represents the main mechanism by which resting HR is increased in chronic hypoxia, whereas the sustained sympathoactivation does not play a major role. Furthermore, our results do not support a contribution of a non-autonomic mechanism. Future studies could investigate whether changes in cardiac muscarinic receptor density or function affect the parasympathetic regulation of HR during longer hypoxic exposure.

References

Bao X, Kennedy BP, Hopkins SR, Bogaard HJ, Wagner PD & Ziegler MG. (2002). Human autonomic activity and its response to acute oxygen supplement after high altitude acclimatization. *Auton Neurosci* **102,** 54-59.

Bender PR, McCullough RE, McCullough RG, Huang SY, Wagner PD, Cymerman A, Hamilton AJ & Reeves JT. (1989). Increased exercise SaO2 independent of ventilatory acclimatization at 4,300 m. *J Appl Physiol (1985)* **66,** 2733-2738.

Boucher M, Chapuy E & Duchenemarullaz P. (1992). Membrane Stabilizing Activity and Beta-Adrenoceptor Antagonist-Induced Bradycardia in Conscious Dogs. *Eur J Pharmacol* **211,** 343-349.

Boushel R, Calbet JA, Radegran G, Sondergaard H, Wagner PD & Saltin B. (2001). Parasympathetic neural activity accounts for the lowering of exercise heart rate at high altitude. *Circulation* **104,** 1785-1791.

Calbet JA. (2003). Chronic hypoxia increases blood pressure and noradrenaline spillover in healthy humans. *J Physiol* **551,** 379-386.

Chapleau MW & Sabharwal R. (2011). Methods of assessing vagus nerve activity and reflexes. *Heart Fail Rev* **16,** 109-127.

Dhar P, Sharma VK, Hota KB, Das SK, Hota SK, Srivastava RB & Singh SB. (2014). Autonomic cardiovascular responses in acclimatized lowlanders on prolonged stay at high altitude: a longitudinal follow up study. *PLoS One* **9,** e84274.

Fujii T, Mori Y, Tominaga T, Hayasaka I & Kawashima K. (1997). Maintenance of constant blood acetylcholine content before and after feeding in young chimpanzees. *Neurosci Lett* **227,** 21-24.

Hansen J & Sander M. (2003). Sympathetic neural overactivity in healthy humans after prolonged exposure to hypobaric hypoxia. *J Physiol* **546,** 921-929.

Hughson RL, Yamamoto Y, McCullough RE, Sutton JR & Reeves JT. (1994). Sympathetic and parasympathetic indicators of heart rate control at altitude studied by spectral analysis. *J Appl Physiol (1985)* **77,** 2537-2542.

Jacobs RA, Siebenmann C, Hug M, Toigo M, Meinild AK & Lundby C. (2012). Twenty-eight days at 3454-m altitude diminishes respiratory capacity but enhances efficiency in human skeletal muscle mitochondria. *FASEB J* **26,** 5192-5200.

Jose AD & Taylor RR. (1969). Autonomic blockade by propranolol and atropine to study intrinsic myocardial function in man. *J Clin Invest* **48,** 2019-2031.

Kacimi R, Richalet JP & Crozatier B. (1993). Hypoxia-induced differential modulation of adenosinergic and muscarinic receptors in rat heart. *J Appl Physiol (1985)* **75,** 1123-1128.

Kato H, Menon AS & Slutsky AS. (1988). Mechanisms mediating the heart rate response to hypoxemia. *Circulation* **77,** 407-414.

Koller EA, Drechsel S, Hess T, Macherel P & Boutellier U. (1988). Effects of atropine and propranolol on the respiratory, circulatory, and ECG responses to high altitude in man. *Eur J Appl Physiol Occup Physiol* **57,** 163-172.

Mazzeo RS, Wolfel EE, Butterfield GE & Reeves JT. (1994). Sympathetic Response during 21 Days at High-Altitude (4,300-M) as Determined by Urinary and Arterial Catecholamines. *Metabolism* **43,** 1226-1232.

Naeije R. (2010). Physiological adaptation of the cardiovascular system to high altitude. *Prog Cardiovasc Dis* **52,** 456-466.

Olshansky B, Sabbah HN, Hauptman PJ & Colucci WS. (2008). Parasympathetic nervous system and heart failure - Pathophysiology and potential implications for therapy. *Circulation* **118,** 863-871.

Richalet JP, Larmignat P, Rathat C, Keromes A, Baud P & Lhoste F. (1988). Decreased cardiac response to isoproterenol infusion in acute and chronic hypoxia. *J Appl Physiol (1985)* **65,** 1957-1961.

Severi S, Cavalcanti S, Mancini E & Santoro A. (2002). Effect of electrolyte and pH changes on the sinus node pacemaking in humans. *J Electrocardiol* **35,** 115-124.

Siebenmann C, Cathomen A, Hug M, Keiser S, Lundby AK, Hilty MP, Goetze JP, Rasmussen P & Lundby C. (2015a). Hemoglobin mass and intravascular volume kinetics during and after exposure to 3,454 m altitude. *J Appl Physiol (1985)***,** jap 01121 02014.

Siebenmann C, Hug M, Keiser S, Muller A, van Lieshout J, Rasmussen P & Lundby C. (2013). Hypovolemia explains the reduced stroke volume at altitude. *Physiol Rep* **1,** e00094.

Siebenmann C & Lundby C. (2015). Regulation of cardiac output in hypoxia. *Scand J Med Sci Sports* **25 Suppl 4,** 53-59.

Siebenmann C, Rasmussen P, Sorensen H, Bonne TC, Zaar M, Aachmann-Andersen NJ, Nordsborg NB, Secher NH & Lundby C. (2015b). Hypoxia increases exercise heart rate despite combined inhibition of beta-adrenergic and muscarinic receptors. *Am J Physiol Heart Circ Physiol* **308,** H1540-1546.

Stembridge M, Ainslie PN, Hughes MG, Stohr EJ, Cotter JD, Nio AQ & Shave R. (2014). Ventricular structure, function, and mechanics at high altitude: chronic remodeling in Sherpa vs. short-term lowlander adaptation. *J Appl Physiol (1985)* **117,** 334-343.

Wesseling KH, Jansen JR, Settels JJ & Schreuder JJ. (1993). Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol* **74,** 2566-2573.

Wolfel EE, Selland MA, Mazzeo RS & Reeves JT. (1994). Systemic hypertension at 4,300 m is related to sympathoadrenal activity. *J Appl Physiol (1985)* **76,** 1643-1650.

476 **Tables**

477 Table 1: Arterial oxygenation, haematocrit and haemoglobin concentration at sea level

478 and at 3,454 m altitude

	Sea level	High altitude	P-value
PaO_2 (mmHg)	91.8 ± 4.2	63.0 ± 1.5	< 0.001
SaO_2 (%)	96. 0 ± 0.4	89.7 ± 0.7	< 0.001
CaO_2 (ml l^{-1})	180 ± 14	185 ± 14	0.036
Haematocrit (%)	42.9 ± 2.6	45.3 ± 3.2	0.008
[Hb] (g l^{-1})	14.0 ± 1.0	15.4 ± 1.3	< 0.001

479 PaO_2, O_2 tension in arterial blood; SaO_2, arterial oxyhaemoglobin saturation; CaO_2, arterial

480 O_2 content; [Hb], haemoglobin concentration in arterial blood.

481

Figure legends and figures

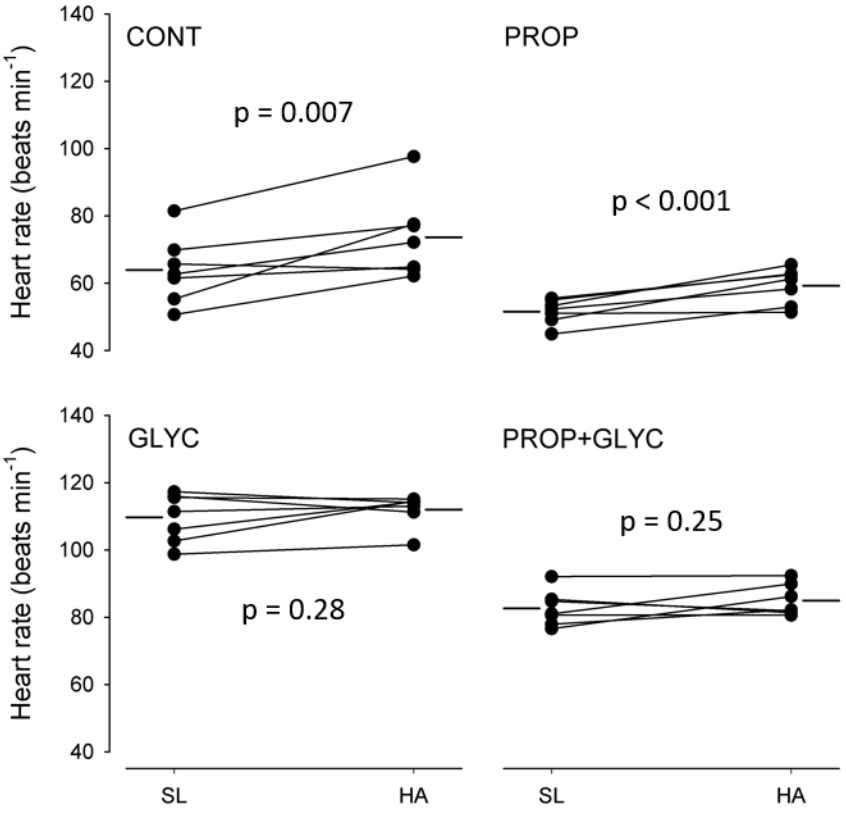
Figure 1. Effect of high altitude exposure on resting heart rate

Points represent individual values and the short horizontal lines the averages at SL and HA, respectively. P-values are given for the comparison between SL and HA within the respective drug condition. SL, sea level; HA, high altitude; CONT, control; PROP, propranolol; GLYC, glycopyrrolate; PROP+GLYC, propranolol and glycopyrrolate in combination.

Figure 2. Effect of high altitude exposure on haemodynamics

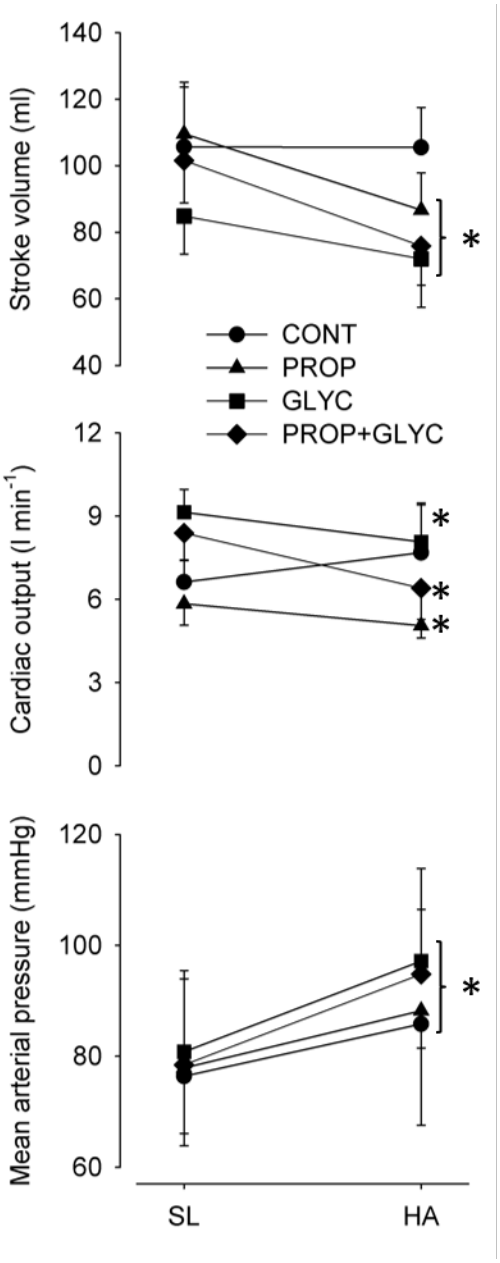
Data points represent means ± S.D. *$p < 0.05$ HA vs. SL within the same drug condition. SL, sea level; HA, high altitude; CONT, control; PROP, propranolol; GLYC, glycopyrrolate; PROP+GLYC, propranolol and glycopyrrolate in combination.

496



497

498 **Figure 1.**



499

500 **Figure 2.**